

CLAIMS

1. Method for detection and/or genetic analysis of HBV in a biological sample, comprising:

- 5 (i) if need be releasing, isolating or concentrating the polynucleic acids present in said sample;
- (ii) if need be amplifying the relevant part of a suitable HBV gene present in said sample with at least one suitable primer pair;
- 10 (iii) hybridizing the polynucleic acids of step (i) or (ii) with a combination of at least two nucleotide probes, with said combination hybridizing specifically to a mutant target sequence chosen from the HBV RT pol gene region and/or to a mutant target sequence chosen from the HBV preCore region and/or to a mutant target sequence chosen from the HBsAg region of HBV and/or to a HBV genotype-specific target sequence, with said target sequences being
- 15 chosen from Figure 1, and with said probes being applied to known locations on a solid support and with said probes being capable of hybridizing to the polynucleic acids of step (i) or (ii) under the same hybridization and wash conditions, or with said probes hybridizing specifically with a sequence complementary to any of said target sequences,
- 20 or a sequence wherein T of said target sequence is replaced by U;
- (iv) detecting the hybrids formed in step (iii);
- (v) inferring the HBV genotype and/or mutants present in said sample from the differential hybridization signal(s) obtained in step (iv).

2. Method according to claim 1, characterized further in that in step (iii) a

25 combination of at least two oligonucleotide probes is used and that said combination of probes hybridizes specifically to at least two of the following groups of target sequences:

a mutant target sequence chosen from the HBV RT pol gene region,

a mutant target sequence chosen from the HBV preCore region,

a mutant target sequence chosen from the HBsAg region of HBV,
a HBV genotype-specific target sequence.

3. Method according to claim 1, characterized further in that in step (iii) a combination of at least three oligonucleotide probes is used and that said combination of probes hybridizes specifically to at least three of the following groups of target sequences:

a mutant target sequence chosen from the HBV RT pol gene region,
a mutant target sequence chosen from the HBV preCore region,
a mutant target sequence chosen from the HBsAg region of HBV,
a HBV genotype-specific target sequence.

4. Method according to claim 1, characterized further in that in step (iii) a combination of at least four oligonucleotide probes is used and that said combination of probes hybridizes specifically to all of the following groups of target sequences:

a mutant target sequence chosen from the HBV RT pol gene region,
a mutant target sequence chosen from the HBV preCore region,
a mutant target sequence chosen from the HBsAg region of HBV,
a HBV genotype-specific target sequence.

5. Method according to any of claims 1 to 4, characterized further in that the oligonucleotide probes used in step (iii) are selected from Table 1, wherein:

-the probes hybridizing specifically to mutant target sequences chosen from the RT pol region of HBV are selected from the following list:

SEQ ID NO 114, SEQ ID NO 115, SEQ ID NO 116, SEQ ID NO 117, SEQ ID NO 127, SEQ ID NO 128, SEQ ID NO 129, SEQ ID NO 130, SEQ ID NO 131, SEQ ID NO 132, SEQ ID NO 133, SEQ ID NO 227, SEQ ID NO 228, SEQ ID NO 229, SEQ ID NO 230, SEQ ID NO 231, SEQ ID NO 232, SEQ ID NO 233, SEQ ID NO 234, SEQ ID NO 243, SEQ ID NO 244, SEQ ID NO 245, SEQ ID NO 246, SEQ ID NO 247, SEQ ID NO 248, SEQ ID NO 249, SEQ ID

NO 250, SEQ ID NO 251, SEQ ID NO 252, SEQ ID NO 253, SEQ ID NO 254, SEQ ID NO 255, SEQ ID NO 256, SEQ ID NO 257, SEQ ID NO 258, SEQ ID NO 259, SEQ ID NO 260, SEQ ID NO 261, SEQ ID NO 262, SEQ ID NO 263, SEQ ID NO 264, SEQ ID NO 265, SEQ ID NO 266, SEQ ID NO 267, SEQ ID NO 268, SEQ ID NO 269, SEQ ID NO 270, SEQ ID NO 271, SEQ ID NO 272, SEQ ID NO 273, SEQ ID NO 274, SEQ ID NO 275, SEQ ID NO 276, SEQ ID NO 277, SEQ ID NO 278, and/or

-the probes hybridizing specifically to mutant target sequences chosen from the preCore region of HBV are selected from the following list:

SEQ ID NO 9, SEQ ID NO 10, SEQ ID NO 11, SEQ ID NO 12, SEQ ID NO 13, SEQ ID NO 34, SEQ ID NO 35, SEQ ID NO 36, SEQ ID NO 37, SEQ ID NO 38, SEQ ID NO 39, SEQ ID NO 40, SEQ ID NO 41, SEQ ID NO 42, SEQ ID NO 43, SEQ ID NO 44, SEQ ID NO 45, SEQ ID NO 46, SEQ ID NO 47, SEQ ID NO 48, SEQ ID NO 49, SEQ ID NO 66, SEQ ID NO 67, SEQ ID NO 68, SEQ ID NO 88, SEQ ID NO 89, SEQ ID NO 90, SEQ ID NO 91, SEQ ID NO 118, SEQ ID NO 119, SEQ ID NO 120, SEQ ID NO 121, SEQ ID NO 122, SEQ ID NO 123, SEQ ID NO 124, SEQ ID NO 125, SEQ ID NO 126, and/or

-the probes hybridizing specifically to mutant target sequences chosen from the HBsAg region of HBV are selected from the following list:

SEQ ID NO 77, SEQ ID NO 78, SEQ ID NO 79, SEQ ID NO 80, SEQ ID NO 81, SEQ ID NO 82, SEQ ID NO 136, SEQ ID NO 137, SEQ ID NO 138, and/or

-the probes hybridizing specifically to genotype-specific target sequences of HBV are selected from the following list:

SEQ ID NO 14, SEQ ID NO 15, SEQ ID NO 16, SEQ ID NO 17, SEQ ID NO 18, SEQ ID NO 19, SEQ ID NO 20, SEQ ID NO 21, SEQ ID NO 22, SEQ ID NO 23, SEQ ID NO 24, SEQ ID NO 25, SEQ ID NO 26, SEQ ID NO 27, SEQ ID NO 28, SEQ ID NO 29, SEQ ID NO 30, SEQ ID NO 31, SEQ ID NO 32,

SEQ ID NO 33, SEQ ID NO 50, SEQ ID NO 51, SEQ ID NO 52, SEQ ID NO
53, SEQ ID NO 54, SEQ ID NO 55, SEQ ID NO 56, SEQ ID NO 57, SEQ ID
NO 58, SEQ ID NO 59, SEQ ID NO 60, SEQ ID NO 61, SEQ ID NO 62, SEQ
ID NO 63, SEQ ID NO 64, SEQ ID NO 65, SEQ ID NO 73, SEQ ID NO 74,
SEQ ID NO 92, SEQ ID NO 93, SEQ ID NO 77, SEQ ID NO 78, SEQ ID NO
79, SEQ ID NO 80, SEQ ID NO 81, SEQ ID NO 82, SEQ ID NO 139, SEQ ID
NO 140, SEQ ID NO 141, SEQ ID NO 142, SEQ ID NO 143, SEQ ID NO
144, SEQ ID NO 145, SEQ ID NO 146, SEQ ID NO 147, SEQ ID NO 148,
SEQ ID NO 149, SEQ ID NO 150, SEQ ID NO 151, SEQ ID NO 152, SEQ ID
NO 153, SEQ ID NO 154, SEQ ID NO 155, SEQ ID NO 156, SEQ ID NO
157, SEQ ID NO 158, SEQ ID NO 159, SEQ ID NO 160, SEQ ID NO 161,
SEQ ID NO 162, SEQ ID NO 163, SEQ ID NO 164, SEQ ID NO 165, SEQ ID
NO 166, SEQ ID NO 167, SEQ ID NO 168, SEQ ID NO 169, SEQ ID NO
170, SEQ ID NO 171, SEQ ID NO 172, SEQ ID NO 173, SEQ ID NO 174,
SEQ ID NO 175, SEQ ID NO 176, SEQ ID NO 177, SEQ ID NO 178, SEQ ID
NO 179, SEQ ID NO 180, SEQ ID NO 181, SEQ ID NO 182, SEQ ID NO
183, SEQ ID NO 184, SEQ ID NO 185, SEQ ID NO 186, SEQ ID NO 187,
SEQ ID NO 188, SEQ ID NO 189, SEQ ID NO 190, SEQ ID NO 191, SEQ ID
NO 192, SEQ ID NO 193, SEQ ID NO 194.

6. Method according to any of claims 1 to 5, wherein the oligonucleotide
probes of step (iii) are characterized in that they specifically hybridize to
target sequences in the RT pol region of HBV and permit detection of
mutations that confer resistance to lamivudine, with said probes being for
instance SEQ ID NO 114, SEQ ID NO 115, SEQ ID NO 116, SEQ ID NO 117,
SEQ ID NO 127, SEQ ID NO 128, SEQ ID NO 129, SEQ ID NO 130, SEQ ID
NO 131, SEQ ID NO 132, SEQ ID NO 133, SEQ ID NO 227, SEQ ID NO
228, SEQ ID NO 229, SEQ ID NO 230, SEQ ID NO 231, SEQ ID NO 232,
SEQ ID NO 233, SEQ ID NO 234, SEQ ID NO 243, SEQ ID NO 244, SEQ ID
NO 245, SEQ ID NO 246, SEQ ID NO 247, SEQ ID NO 248, SEQ ID NO
249, SEQ ID NO 250, SEQ ID NO 251, SEQ ID NO 252, SEQ ID NO 253,

SEQ ID NO 254, SEQ ID NO 255, SEQ ID NO 256, SEQ ID NO 257, SEQ ID NO 258, SEQ ID NO 259, SEQ ID NO 260, SEQ ID NO 261, SEQ ID NO 262, SEQ ID NO 263, SEQ ID NO 264, SEQ ID NO 265, SEQ ID NO 266, SEQ ID NO 269, SEQ ID NO 270, SEQ ID NO 271, SEQ ID NO 272, SEQ ID NO 275, SEQ ID NO 276, SEQ ID NO 277, SEQ ID NO 278.

7. Method according to any of claims 1 to 5, wherein the oligonucleotide probes of step (iii) are characterized in that they specifically hybridize to target sequences in the RT pol region of HBV and permit detection of mutations that confer resistance to penciclovir, with said probes being for instance SEQ ID NO 267, SEQ ID NO 268, SEQ ID NO 269, SEQ ID NO 270, SEQ ID NO 271, SEQ ID NO 272, SEQ ID NO 273, SEQ ID NO 274, SEQ ID NO 275, SEQ ID NO 276, SEQ ID NO 277, SEQ ID NO 278.

8. Method according to any of claims 1 to 5, wherein the oligonucleotide probes of step (iii) are characterized in that they specifically hybridize to target sequences in the RT pol region of HBV and permit detection of at least one of the mutations that give rise to a change of the following amino acids: F at position 514, V at position 521, P at position 525, L at position 527, M at position 552, V at position 555, with said probes being for instance SEQ ID NO 114, SEQ ID NO 115, SEQ ID NO 116, SEQ ID NO 117, SEQ ID NO 127, SEQ ID NO 128, SEQ ID NO 129, SEQ ID NO 130, SEQ ID NO 131, SEQ ID NO 132, SEQ ID NO 133, SEQ ID NO 227, SEQ ID NO 228, SEQ ID NO 229, SEQ ID NO 230, SEQ ID NO 231, SEQ ID NO 232, SEQ ID NO 233, SEQ ID NO 234, SEQ ID NO 243, SEQ ID NO 244, SEQ ID NO 245, SEQ ID NO 246, SEQ ID NO 247, SEQ ID NO 248, SEQ ID NO 249, SEQ ID NO 250, SEQ ID NO 251, SEQ ID NO 252, SEQ ID NO 253, SEQ ID NO 254, SEQ ID NO 255, SEQ ID NO 256, SEQ ID NO 257, SEQ ID NO 258, SEQ ID NO 259, SEQ ID NO 260, SEQ ID NO 261, SEQ ID NO 262, SEQ ID NO 263, SEQ ID NO 264, SEQ ID NO 265, SEQ ID NO 266, SEQ ID NO 267, SEQ ID NO 268, SEQ ID NO 269, SEQ ID NO 270, SEQ ID

NO 271, SEQ ID NO 272, SEQ ID NO 273, SEQ ID NO 274, SEQ ID NO 275, SEQ ID NO 276, SEQ ID NO 277, SEQ ID NO 278.

9. Method according to any of claims 1 to 5, characterized further in that the probes of step (iii) hybridize specifically to a genotype-specific target sequence from the HBsAg region, said probes being for instance SEQ ID NO 77, SEQ ID NO 78, SEQ ID NO 79, SEQ ID NO 80, SEQ ID NO 81, SEQ ID NO 82, SEQ ID NO 139, SEQ ID NO 140, SEQ ID NO 141, SEQ ID NO 142, SEQ ID NO 143, SEQ ID NO 144, SEQ ID NO 145, SEQ ID NO 146, SEQ ID NO 147, SEQ ID NO 148, SEQ ID NO 149, SEQ ID NO 150, SEQ ID NO 151, SEQ ID NO 152, SEQ ID NO 153, SEQ ID NO 154, SEQ ID NO 155, SEQ ID NO 156, SEQ ID NO 157, SEQ ID NO 158, SEQ ID NO 159, SEQ ID NO 160, SEQ ID NO 161, SEQ ID NO 162, SEQ ID NO 163, SEQ ID NO 164, SEQ ID NO 165, SEQ ID NO 166, SEQ ID NO 167, SEQ ID NO 168, SEQ ID NO 169, SEQ ID NO 170, SEQ ID NO 171, SEQ ID NO 172, SEQ ID NO 173, SEQ ID NO 174, SEQ ID NO 175, SEQ ID NO 176, SEQ ID NO 177, SEQ ID NO 178, SEQ ID NO 179, SEQ ID NO 180, SEQ ID NO 181, SEQ ID NO 182, SEQ ID NO 183, SEQ ID NO 184, SEQ ID NO 185, SEQ ID NO 186, SEQ ID NO 187, SEQ ID NO 188, SEQ ID NO 189, SEQ ID NO 190, SEQ ID NO 191, SEQ ID NO 192, SEQ ID NO 193, SEQ ID NO 194, SEQ ID NO 214, SEQ ID NO 215, SEQ ID NO 216, SEQ ID NO 217, SEQ ID NO 218, SEQ ID NO 219.

10. Method according to any of claims 1 to 5, characterized further in that the probes of step (iii) hybridize specifically to a genotype-specific target sequence from the preS1 region, said probes being for instance SEQ ID NO 14, SEQ ID NO 15, SEQ ID NO 16, SEQ ID NO 17, SEQ ID NO 18, SEQ ID NO 19, SEQ ID NO 20, SEQ ID NO 21, SEQ ID NO 22, SEQ ID NO 23, SEQ ID NO 24, SEQ ID NO 25, SEQ ID NO 26, SEQ ID NO 27, SEQ ID NO 28, SEQ ID NO 29, SEQ ID NO 30, SEQ ID NO 31, SEQ ID NO 32, SEQ ID NO 33, SEQ ID NO 50, SEQ ID NO 51, SEQ ID NO 52, SEQ ID NO 53, SEQ ID

NO 54, SEQ ID NO 55, SEQ ID NO 56, SEQ ID NO 57, SEQ ID NO 58, SEQ ID NO 59, SEQ ID NO 60, SEQ ID NO 61, SEQ ID NO 62, SEQ ID NO 63, SEQ ID NO 64, SEQ ID NO 65, SEQ ID NO 73, SEQ ID NO 74, SEQ ID NO 92, SEQ ID NO 93.

- 5 11. A composition comprising at least one probe as defined in any of claims 1 to 10.
12. A composition comprising at least one probe as defined in claim 5.
13. Use of a composition of probes as defined in claims 11 and/or 12 for *in vitro* diagnosing and/or monitoring HBV mutants and/or genotypes present in a biological sample.
- 10 14. Assay kit for the detection and/or the genetic analysis of HBV mutants and/or genotypes present in a biological sample according to the method of any of claims 1 to 10, comprising the following components :
- 15 (i) when appropriate, a means for releasing, isolating or concentrating the polynucleic acids present in said sample;
- (ii) when appropriate, at least one suitable primer pair;
- (iii) at least one of the probes according to claim 11 and/or 12, possibly fixed to a solid support;
- (iv) a hybridization buffer, or components necessary for producing said buffer;
- 20 (v) a wash solution, or components necessary for producing said solution;
- (vi) when appropriate, a means for detecting the hybrids resulting from the preceding hybridization.
- (vii) when appropriate, a means for attaching said probe to a known location on a solid support.